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Discussion

Dr Brendan Stiles *(New York, NY).* Farshad, that was nicely presented. It is a good continuation of the work your group has done. I am a bit curious why you didn't use a sham experiment on the knockout mice. Or did you do that?

Dr Anvari. Our preliminary studies on these knockout mice showed that they are comparable to our WT mice in terms of pulmonary function, and we saw similar measurements as the WT mice. So we did not include 2 sham groups in these experiments.

Dr Stiles. So the baseline levels of cytokine expression, things like that, are no different between the knockout and the sham?

Dr Anvari. The baseline levels were similar for function. As far as cytokines, previous studies have shown that the baseline levels of some of the cytokines, including TNF-α and IL-6 are elevated in the knockout mice. So we did not repeat those experiments.

Dr Stiles. I am just concerned that you are knocking out a proinflammatory gene and then you are showing that there is a decrease in cytokine induction after IR, which you attribute to the treatment. Some of those things are downstream of the proinflammatory pathway that has been knocked out. I am concerned that what you are showing is more just a function of knocking that out rather than actual protection from IR. Do you think there is a way to tease that out?

Dr Anvari. This is an interesting question. The limitation with knockouts, as you mentioned, is that the observed effects may be the result of the creation of the knockout itself. We do know that the expression of other adenosine receptors are unchanged in these knockouts. I would look at this as a first step. We recently acquired agonists and antagonists for this receptor, and I think that will be the next step to validate these results. Our preliminary results look promising.

Dr Stiles. That is great. What is the delivery method of those, the agonists and antagonists?

Dr Anvari. Intravenous injections via the external jugular.

Dr Frank Sellke *(Providence, RI).* I have a technical question. You used a *t* test for your statistical analysis, yet you had multiple groups. Shouldn't you use a multiple comparison test rather than a *t* test?

Dr Anvari. We performed an analysis of variance (ANOVA) followed by a *t* test for unpaired data.

Dr Sellke. Yes, but if you have multiple groups, you have to take the multiple comparisons into consideration, not just an ANOVA. You have to do a post hoc analysis with multiple comparisons like a Bonferroni correction or one of these tests into play.

Dr Anvari. Correct. We did an ANOVA in conjunction with a Tukey's test. We also performed additional analyses by looking at specific groups comparing 2 at a time using a *t* test.

Dr Sellke. So you are not really doing a Bonferroni correction if you are doing just a *t* test.

Dr Anvari. No. ANOVA in conjunction with Tukey's test. The *t* test was used for additional analysis.

Dr Michael Jessen *(Dallas, Tex).* I enjoyed your study a lot. That is good work. The biggest area in cardiothoracic surgery I think where we see IR injury, as you pointed out, is in lung transplantation, but it is a tough thing to model. In some ways your model deviates from it. There is no denervation, no hypothermia, no preservation solution, and no immunosuppression on board. How confident are you that your findings will be maintained in a setting that is more clinically relevant to lung transplantation?

Dr Anvari. Those are all very relevant issues. We adopted this model as a first step in the process of better understanding the role of this receptor in pulmonary IR. We chose a mouse model because we had the benefit of the knockouts, and this provides a foundation. We need to continue this work by using drugs in this model, and if that confirms the results we are seeing, then we can take that to a bigger animal and use a more clinically relevant model. But I think this is an important first step in establishing that.

This in vivo model is, of course, as you mentioned, a warm ischemia model and doesn't take into account all the factors you mentioned, but in the mouse is the best model short of actual transplantation, which has recently been done. But, as you can imagine, it is technically challenging and not easily reproducible.

Dr Glen Van Arsdell *(Toronto, Ontario, Canada).* Your group has been working with adenosine receptors for some time. The program also does lung transplantation. Have you taken human tissue to see whether there is a difference in expression and correlated that with IR that you see clinically?

Dr Anvari. We have not used any human tissue in our experiments so far.

Dr Van Arsdell. Is that coming down the line?

Dr Anvari. Hopefully, in the near future.